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Hypothesis: Transgene establishment in wild relatives of wheat can be prevented by utilizing the *Ph*1 gene as a *senso stricto* chaperon to prevent homoeologous recombination

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ABSTRACT

Durum and bread wheat need transgenic traits such as herbicide and disease resistance due to recent evolution of herbicide resistant grass weeds and an intractable new strain of stem rust. Transgenic wheat varieties have not been commercialized partly due to potential transgene movement to wild/weedy relatives, which occurs naturally to closely related *Aegilops* and other spp. Recombination does not occur in the F_1 hybrid between wheat and its relatives due to the presence of the *Ph*1 gene on wheat chromosome arm 5BL, which acts as a chaperone, preventing promiscuous homoeologous pairing to similar, but not homologous chromosomes of the wild/weedy species. Thus recombination must occur during backcrossing after the wheat *Ph*1 gene has been eliminated. Based on these findings, we speculate that *Ph*1 could be used to prevent gene introgression into weedy relatives. We propose two methods to prevent such transgene establishment: (1) link the transgene in proximity to the wheat *Ph*1 gene and (2) insert the transgene in tandem with the lethal *barnase* on any chromosome arm other than 5BL, and insert *barstar*, which suppresses barnase on chromosome arm 5BL in proximity to *Ph*1. The presence of *Ph*1 in backcross plants containing 5BL will prevent the homoeologous establishment of *barnase* coupled to the desired transgene in the wild population. 5BL itself will be eliminated during repeated backcrossing to the wild parent, and progeny bearing the desired transgene in tandem with *barnase* but without the *Ph*1-*barstar* complex will die.

1. Introduction

Farmers need transgenic crop varieties to deal with problems where breeding cannot help, or where breeding is slow. Weed control, for example, is one problem where breeding has been of little assistance [1], but transgenic crops have opened new horizons for weed control. With the growing use of transgenic crops in recent years, there has been a growing concern about gene introgression from wheat into related genera. Grass weeds have evolved resistance to all wheat-selective herbicides, and the control options available can only come from transgenics [2–7]. A new strain of wheat stem rust threatens wheat production worldwide, and no wheat genes have been identified that confer resistance [8,9]. Transgenically reducing or modifying the lignin in wheat straw by suppressing lignin biosynthesis genes could provide efficient fodder for ruminants or a feedstock for biofuels, far easier than screening for recessive loss of function genes in this hexaploid crop [10], but gene flow issues preclude considering this.

Surprisingly, despite the need for information, there are very few studies describing introgression from wheat into related wild species, especially regarding introgression between homoeologous (non-homologous) genomes [11–19]. A chaperon[1] of transgenes is needed to protect against such promiscuous homoeologous recombination.

As transgenics are imperative to the future of wheat [2,3] and other polyploid crops, the issue of transgene flow to related weeds and wild species must be dealt with [3,16,17]. Chromosomal gene placement has been suggested as a possible failsafe mechanism for transgenic wheat; it was speculated that placing the transgene on a wheat genome not shared by the neighboring wild or weedy relatives, could reduce the risk of transgene movement (compared to genes on homologous chromosomes) [3].

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[1] Oxford dictionary: "chaperone (n): a person, especially a married elderly person, who for the sake of propriety, accompanies a young unmarried lady in public as a guide and protector" (i.e. prevents promiscuous recombination).

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Homoeologous chromosomes rarely pair in the F_1 progeny of crosses between wheat and related species, due to the presence of the *Ph*1 gene, which acts as a chaperone by suppressing homoeologous pairing in wheat. *Ph*1 is located on the long arm of chromosome 5B [20–22]. This gene, when present in doses of one (as in the case in wide hybrids) or two, suppresses only homoeologous pairing, but in a dose of six it suppresses all pairing, including homologous pairing [23]. Although not previously demonstrated or discussed, it seems logical that wheat gene introgression into homoeologous chromosomes of relatives would have to occur in backcrosses or selfed progeny after segregation and disappearance from the progeny of chromosome 5B, allowing homoeologous introgression of the transgene.

We have shown that there has been homoeologous gene flow from wheat into *Aegilops peregrina* in the field, under natural conditions [11]. We now present experimental evidence for gene transfer from *Triticum aestivum* into the homoeologous genomes of *Ae. peregrina* at a frequency that exceeds the frequency of homoeologous pairing in the F_1 hybrid between these two species. Our results support the contention that introgression predominantly occurs in backcross or selfed progeny that do not contain the *Ph*1 gene. Based on these results, we propose a useful method to prevent promiscuous transgene flow from polyploid crops to their wild/weedy relatives.

2. Materials and methods

2.1. Plant material

Ae. peregrina accession TKE02 (female) was crossed with *T. aestivum* cv. Chinese Spring (male). Two hybrid plants (F_1) were backcrossed three times to *Ae. peregrina* to produce backcross generations one, two, and three (BC_1F_1, BC_2F_1, BC_3F_1). BC_3F_1 plants having 14 bivalents and 1 to several univalents were allowed to self to produce generations BC_3F_1. Each potential female parent was emasculated in all crosses and surrounded in close proximity by several plants of *Ae. peregrina* and bagged after pollination. All plant tillers were shaken several times a day to ensure a good distribution of pollen. Hybrid and backcross plants were grown in 3-L pots in a greenhouse with 16-h light per day at 20 °C. *Ae. peregrina* plants were grown under the same conditions, but in 10-L pots to allow them to reach maximal size and a large number of tillers. This was important for the production of large amounts of pollen.

2.2. Amplified fragment length polymorphism (AFLP) analysis

AFLP was performed according to [24] with the following modifications: (1) the primers were labeled with one of the fluorescent dyes FAM (6-carboxyfluorescein), HEX (4,7,2′,4′,5′,7′-hexachloro-6-carboxyfluorescein), or TET (4,7,2′,7′-tetrachloro-6-carboxyfluorescein) (Eisenberg Bros. Ltd.). (2) At the end of the electrophoresis, the gels were scanned with a Typhoon™ 9400 scanner (Amersham Bioscience) with the appropriate lasers, at medium sensitivity. (3) The intensity of the digital image of the gel image was then adjusted using the ImageQuant™ software (supplied with the scanner). Ten primer combinations were randomly chosen for the analysis. The selective nucleotides and the primer dyes are listed in Table 1.

We determined which genome of wheat probably contributed the DNA of the band that appeared according to the following three criteria: (1) bands that are present in *T. aestivum* (genome BBAADD), and *T. urartu* (AA), present in *T. turgidum* ssp. *durum* (AABB), but missing from *Ae. tauschii* (DD) and thus are probably located on the A genome of *T. aestivum*; (2) bands that are present in *T. aestivum* (BBAADD) and *T. turgidum* ssp. *durum* (AABB), but missing from *T. urartu* (AA), and *Ae. tauschii* (DD) and thus are probably located on the B genome of *T. aestivum*, and; (3) bands that are present in *T. aestivum* (BBAADD) and *Ae. tauschii* (DD), but missing from *T. turgidum* ssp. *durum* (AABB) and *T. urartu* (AA) and thus are probably located on the D genome of *T. aestivum*. Introgressed bands that did not fall into any of the three categories were defined as bands whose genomic origin in wheat could not be determined.

Table 1
The 10 primer combinations that were randomly chosen to detect introgression from *Triticum aestivum* into *Aegilops peregrina* by AFLP

MseI selective nucleotides	EcoRI selective nucleotides
CTG	ACT (FAM)
CTT	ACA (TET)
CTT	AAC (HEX)
CTT	ACC (FAM)
CAG	ACA (TET)
CAT	ACA (TET)
CTA	ACA (TET)
CTC	ACA (TET)
CAG	ACC (FAM)
CAG	ACG (HEX)

The dye of the EcoRI primers is in brackets.

2.3. Cytogenetic analysis of hybrids and successive backcross generations

Root tips from hybrid and backcross seedlings were collected and placed in vials containing cold water, placed in crushed ice, refrigerated for 30 h and then transferred into the fixative solution of 3 ethanol:1 acetic acid. The fixative solution was changed several times during the first week. The material was kept at 4 °C until analyzed. Anthers from mature plants were placed immediately after collection in the fixative solution. Fixed root tips or anthers were placed on a glass slide with a drop of 2% acetocarmine, heated over a moderate flame for several seconds and then squashed. The number of chromosomes (at mitosis) and bivalents (at meiosis) was determined by examination of the slides with an Olympus inverted microscope IX, and photographed by an Imago CCD camera with TiLLvisION 3.3 software (T. I. I. L Photonics).

3. Results and discussion

3.1. Wheat genes do homoeologously introgress into a wild species

In order to determine the frequency of gene transfer from wheat into the homoeologous genomes of a wild related species, we traced the backcross progeny of a single cross between *Ae. peregrina*, accession TKE02 (genome S^vS^vUU), and *T. aestivum* cv. Chinese Spring (genome BBAADD). We randomly chose 15 BC_3F_2 plants for the analysis that had the stabilized chromosome number of $2n = 28$ of *Ae. peregrina* (Fig. 1A and B) that also phenotypically resembled *Ae. peregrina* (data not shown). The frequency of introgression of random DNA segments from the genomes of *T. aestivum* into the homoeologous genomes of *Ae. peregrina* was determined by amplified fragment length polymorphism (AFLP) and 528 clear bands were analyzed (Fig. 2). Bands that were present in *T. aestivum* F_1 and BC_3F_2 plants but not found in *Ae. peregrina* were defined as introgressed bands. Seventeen AFLP fragments introgressed from *T. aestivum* into *Ae. peregrina* (Table 2 and Fig. 2): two bands from the B sub-genome, two bands from the A sub-genome, and seven from the D sub-genome. The probable wheat sub-genome origin of six